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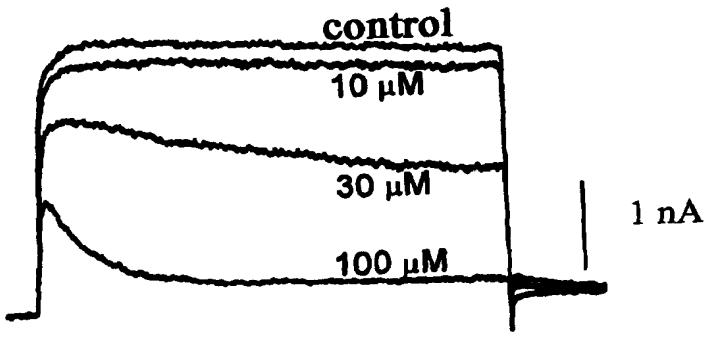
(54) Title: PHARMACEUTICAL COMPOSITIONS COMPRISING CHELIDONINE OR DERIVATIVES THEREOF



(57) Abstract: The present invention relation to a pharmaceutical composition comprising chelidonine or derivatives thereof, with pharmaceutically acceptable carriers. The coompositiions according to the present invention can selectively block hKv1.5 channels expressed preferentially in human atrial myocytes, and thus are useful as K+channel blockeers and antiarrhythmic drugs.

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Pharmaceutical compositions comprising chelidonine or derivatives thereof

Technical Field

The present invention relates to a composition comprising chelidonine or derivatives thereof, with pharmaceutically acceptable carriers.

Background Art

Arrhythmias are abnormal rhythms of the heart and cause the heart to pump less effectively. At this time, electro-biochemical properties on local regions of cardiac muscle are changed due to a variety of causes, and thus abnormal cardiac impulse formation or impulse propagation occurs (www.americanheart.org/heart and stroke).

The shape and duration of cardiac action potentials vary depending on the region of the heart where they are recorded. These regional differences result, in part, from the differential expression of K^+ channel genes within the myocardium (Sanguinetti and Keating. Role of delayed rectifier potassium channels in cardiac repolarization and arrhythmias. *News Physiol Sci* 1997, 12:152-157).

All antiarrhythmic drugs influence the movement of ions in cardiac muscle to exhibit antiarrhythmic effects. Accordingly, antiarrhythmic drugs are commonly divided into the following classes according to the kinds of the moving ions: I (sodium channel blockers), II (ß-adrenergic receptor blockers), III (potassium channel blockers), IV (calcium channel blockers), etc. (Katz AM. Selectivity and toxicity of antiarrhythmic drugs: molecular interactions with ion channels. *AM J Med* 1998, 104: 179-195). A major obstacle to the widespread use of drugs to manage cardiac arrhythmias has been a relatively high incidence of extracardiac side effects. With increasingly sophisticated drug development, it is possible to develop drugs that show significantly reduced extracardiac side effects due to the improved tissue-specificity. However, cardiac side effects, which often arise as a direct consequence of drugs' antiarrhythmic mechanisms, have been very difficult to be circumvented. Common cardiac side effects of antiarrhythmic drugs include depressed contractile performance, bradycardia, altered

efficacy of pacing and defibrillating devices, and the occurrence of new arrhythmias or increased occurrence of arrhythmias (proarrhythmia) (Roden DM. Mechanism and management of proarrhythmia. *Am J Cardiol* 1998, 82: 491-571).

Antiarrhythmic drugs regulating action potential durations, which are important in controlling heart rate, have already been developed. However, these drugs also produce various side effects as described above, which limits their clinical applications.

Therefore, an ideal antiarrhythmic drug with fewer side effects must act only on cardiac myocytes showing abnormal excitability (or cells having abnormal heart rate), or arrhythmia-occurring tissues (e.g., atrial myocytes, ventricular myocytes, Purkinje fibers, etc). However, drugs satisfying the above requirements have not yet been developed. In order to develop a novel drug with few or no side effects, molecular biological knowledge for the targets of antiarrhythmic drugs (such as ion channels) must be accompanied. For example, the ion channel which selectively expresses in arrhythmogenic tissues, is one of the targets of ideal antiarrhythmic drugs. Accordingly, approaches by the combination of molecular biological cloning techniques and electro-pharmacological techniques will make it possible to develop new ideal antiarrhythmic drugs.

It is well known that various K^+ channels regulate action potential durations and K^+ channel genes differentially express depending on the regions of the heart.

K^+ channels represent the most diverse class of ion channels in heart. K^+ currents in the myocardium can be classified into two categories: 1) inward K^+ currents such as I_{K1} (inward rectifying K^+ current), I_{KAch} (acetylcholine-activated K^+ current), and I_{KATP} (ATP-sensitive K^+ current); and 2) voltage-gated K^+ (Kv) currents. The inward K^+ currents regulate resting membrane potential, whereas the Kv currents control action potential duration.

The cardiac Kv currents are divided into I_{to}, I_{KP}, I_{KR}, I_{KUR}, and I_{KS} in accordance with their electrophysiological characteristics. I_{to} current, a transient outward K^+ current, is activated immediately after membrane depolarization, and then becomes inactive rapidly. Therefore, I_{to} is of importance in phase 1 of action potential. I_{KP} current, a plateau K^+ current, becomes active only during membrane depolarization, and is a kind of delayed outward K^+ current with an intermediate rate of activation.

I_{KR} current, a rapidly activating delayed rectifier K^+ current, is of importance in phase 2 of action potential. I_{KUR} current, an ultra-rapidly activating delayed rectifier K^+ current, is also of importance in phase 2 of action potential. I_{KS} current, a slow-activating delayed rectifier K^+ current, takes a few seconds to become active completely, and is of importance in final repolarization of phase 3 of action potential (Roden and George, The cardiac ion channel: relevance to management of arrhythmias. *Annu Rev Med* 1999, 47: 138-148). These Kv channels contribute to cell repolarization and regulate the action potential duration. Clinically, it is known that the repolarization disorders in the damaged tissues result in cardiac arrhythmias. Accordingly, Kv channels become major targets for the treatment of arrhythmias. In practical use, it is known that antiarrhythmic drugs such as quinidine, verapamil, nifedipine, sotalol, amiodarone, flecainide, and cropyrium interact with the Kv channels (Katz AM. Selectivity and toxicity of antiarrhythmic drugs: molecular interactions with ion channels. *Am J Med* 1998, 104: 174-195). However, these drugs are known to have various side effects due to their lack of selectivity for ion channels. Accordingly, there remains a need to develop a novel drug acting specifically on the ion channel in extraordinarily hyperexcitable tissues.

The first cloned K^+ channel gene, *Shaker*, was obtained using the techniques of *Drosophila* genetics and DNA manipulation. cDNAs of mammalian Kv channel reported until now are divided into nine subfamilies, Kv1~Kv9. Among them, Kv1 subfamily is the most diverse one, and includes at least eight subclasses, Kv1.1~Kv1.8 (Grissner S. Potassium channels still hot, TiPS 1997, 18: 347-350). Kv1.1, Kv1.2, Kv1.4, Kv1.5, Kv2.1, Kv4.2 and Kv4.3 of Kv channel genes have been cloned from cardiac tissue (Deal, *et al.*, Molecular physiology of cardiac potassium channels. *Physiol Rev* 1996, 76: 49-67). Main Kv channel genes expressed in human heart are hKv1.4, hKv1.5, hKv4.3 and HERG genes. All these genes are highly expressed in both atrium and ventricle, and in particular, the hKv1.5 gene is preferentially expressed in human atrium. The hKv1.5 is known to have the same electrophysiological and pharmacological properties as I_{KUR}, a current specific in human atrium (Fedid, *et al.*, The 1997 Stevenson Award Lecture, Cardiac K^+ channel gating: cloned delayed rectifier mechanisms and drug modulation. *Can J Physiol Pharmacol* 1998, 76: 77-89). Development of highly selective blockers for the hKv1.5 channel will lead to an ideal

drug for the treatment of atrial fibrillations.

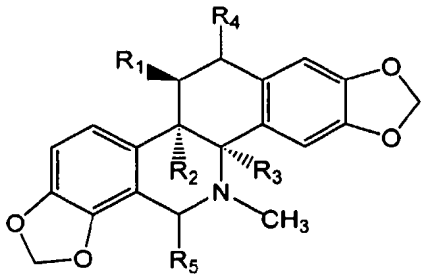
The present inventors have earnestly and intensively searched to develop a selective blocker for the hKv1.5 channel which is preferentially expressed in human atrium, and as a result, have found that chelidonine and derivatives thereof inhibit hKv1.5 channel currents and I_{KUR} currents in human atrial myocytes. In addition, they also found that the prolonging effects of action potential duration are proportional to the heart rate.

Therefore, it is an object of the present invention to provide a composition comprising chelidonine or derivatives thereof which exhibit excellent K^+ channel blocking effect and antiarrhythmic effect, with pharmaceutically acceptable carriers.

Disclosure of the Invention

The present invention relates to a composition comprising chelidonine or derivatives thereof represented by the following formula 1, with pharmaceutically acceptable carriers:

【Formula 1】



wherein,

R_1 is selected from the group consisting of hydrogen, hydroxy, a lower alkoxy having 1 to 5 carbon atoms, benzyloxy, a lower alkylcarbonyloxy having 1 to 5 carbon atoms, benzoyloxy, a lower alkylsulfonyloxy having 1 to 5 carbon atoms, arylsulfonyloxy, diphenylphosphonyloxy and -$OCONH_2$;

R_2 is hydrogen or methyl; and

R_3, R_4 and R_5 are each, independently, hydrogen; or

R_1 forms a double bond with R_2 or R_4; or

R_2 forms a double bond with R_3; or

R_5 forms a double bond with the adjacent N atom.

Preferred compositions according to the present invention comprise chelidonine or derivatives thereof:

wherein,

R_1 is selected from the group consisting of hydrogen, hydroxy, methoxy, benzyloxy, acetoxy, benzoyloxy, methylsulfonyloxy, 4-methyl-benzenesulfonyloxy, diphenylphosphonyloxy and -$OCONH_2$;

R_2 is hydrogen or methyl; and

R_3, R_4 and R_5 are each, independently, hydrogen; or

R_1 forms a double bond with R_2 or R_4; or

R_2 forms a double bond with R_3; or

R_5 forms a double bond with the adjacent N atom.

More preferred compositions according to the present invention comprise one selected from the group consisting of:

[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridin-6-ol;

[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-6-methoxy-13-methyl[1,3]-benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridine;

[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-6-benzyloxy-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridine;

{[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridin-6-yl}-acetate;

{[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridin-6-yl}-benzoate;

(12bR)-13,14-dihydro-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridine;

(12bR)-7, 12b, 13, 14-tetrahydro-13-methyl[1,3]-benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridine;

[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-

methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridine;

{[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridin-6-yl}-diphenylphosphate;

{[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridin-6-yl}-methanesulfonate;

{[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridin-6-yl}-4-methylbenzenesulfonate;

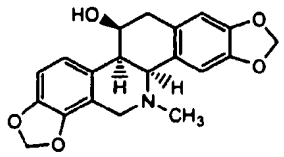
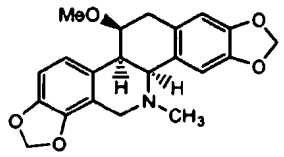
{[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridin-6-yl}-carbamate;

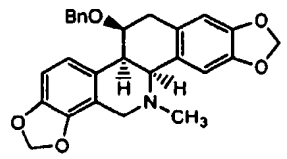
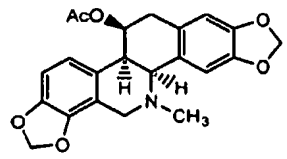
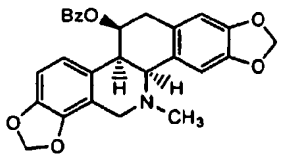
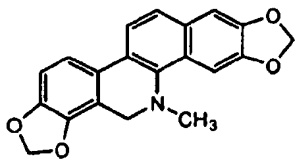
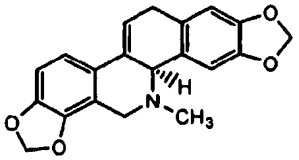
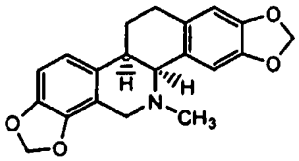
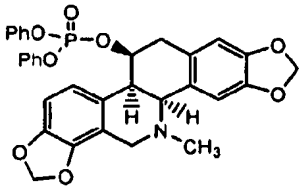
[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-5b, 13-dimethyl[1,3]-benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridin-6-ol; and

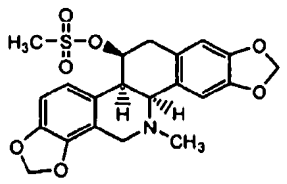
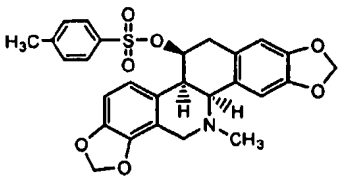
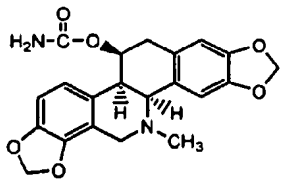
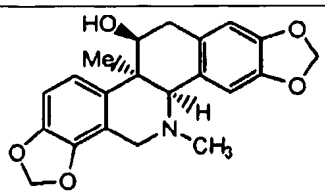
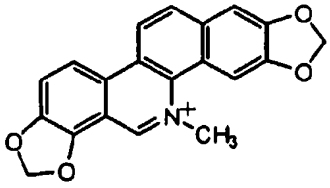
13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridinium.

The structural formulae of the compounds as described above are shown in the following Table 1:

【Table 1】

Compound No.	Formula	Compound
1		[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridin-6-ol
2		[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-6-methoxy-13-methyl[1,3]-benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridine

3		[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-6-benzyloxy-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridine
4		{[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3] benzodioxolo[5,6c]-1,3-dioxolo[4,5-i] phenanthridin-6-yl}-acetate
5		{[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3] benzodioxolo[5,6c]-1,3-dioxolo[4,5-i] phenanthridin-6-yl}-benzoate
6		(12bR)-13,14-dihydro-13-methyl [1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridine
7		(12bR)-7, 12b, 13, 14-tetrahydro-13-methyl[1,3]–benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridine
8		[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3] benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridine
9		{[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3] benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridin-6-yl}-diphenylphosphate

10		{[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3] benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridin-6-yl}-methanesulfonate
11		{[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3] benzodioxolo[5,6c]-1,3-dioxolo[4,5-i] phenanthridin-6-yl}-4-methylbenzenesulfonate
12		{[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14–hexahydro-13-methyl[1,3] benzodioxolo[5,6c]-1,3-dioxolo[4,5-i] phenanthridin-6-yl}-carbamate
13		[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-5b,13-dimethyl[1,3]-benzodioxolo[5,6c]-1,3-dioxolo[4,5-i] phenanthridin-6-ol
14		13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridinium

Chelidonine of compound **1** used as an effective ingredient in the present invention is mainly present in plants. Chelidonine has few problems with regard to safety and toxicity. It is well known that chelidonine exhibits anti-spasm effects, inhibitory effects against central dopamine, analgesic effects, antipyretic effects, anti-cancer effects, and inhibitory effects against nitric oxide, corn and verruca.

Compounds 8, 9, 11 and 12 included in the composition of the present invention are novel compounds. On the other hand, compounds other than these compounds have been already disclosed in the literatures below.

Compound 1

- Simanek, V. Benzophenanthridine alkaloids. In: The Alkaloids. Academic Press. 1985, Vol. 4, 185-240;

Compound 2

- Nowicky, Wassili. Carcinostatic agents and their use. Patent 1,191,837, 1985, 8, 13, Canada;

Compound 3

- Grynkiewicz, Grzegorz; Chojecka-Koryn, Ewa, Gadzikowska, Maria; Chodkowska, Anna, Jagiello-Wojtowicz, Ewa, Synthesis and biological activity of O-acyl and O-alkyl chelidonine derivatives. *Eur J Med Chem.* 2001, 36:951-960;

Compound 4

- Grynkiewicz, Grzegorz; Chojecka-Koryn, Ewa, Gadzikowska, Maria; Chodkowska, Anna, Jagiello-Wojtowicz, Ewa, Synthesis and biological activity of O-acyl and O-alkyl chelidonine derivatives. *Eur J Med Chem.* 2001, 36:951-960;

Compound 5

- Grynkiewicz, Grzegorz; Chojecka-Koryn, Ewa, Gadzikowska, Maria; Chodkowska, Anna, Jagiello-Wojtowicz, Ewa, Synthesis and biological activity of O-acyl and O-alkyl chelidonine derivatives. *Eur J Med Chem.* 2001, 36:951-960;

Compound 6

- Hanaoka, Miyoji; Yoshida, Shuji; Annen, Masami; Mukai, Chisato., A novel and biomimetic synthesis of (±)-chelamine, (±)-chelidonine, sanguinarine, and dihydrosanguinarine from coptisine via a common intermediate. *Chem Lett.* 1986, 5:739-742;

Compound 7

- Snatzke, Guenther; Hrbek, Jaroslav, Jr.; Hruben, Ladislav; Horeau, Alain; Santavy, Frantisek, Circular dichroism. XLII. Isolation and chemistry of the alkaloids from some plants of the genus Papaver. L. III. Absolute configuration and chiroptical properties of chelidonine and tetrahydroberberine alkaloids. *Tetrahedron* 1970, 26(21):5013-5028;

Compound 10

- Grynkiewicz, Grzegorz; Chojecka-Koryn, Ewa, Gadzikowska, Maria; Chodkowska, Anna, Jagiello-Wojtowicz, Ewa, Synthesis and biological activity of O-acyl and O-alkyl chelidonine derivatives. *Eur J Med Chem.* 2001, 36:951-960;

Compound 13

Takao, Narao A. Alkaloids of Papaveraceae. VI. Alkaloids of Corydalis incisa. 5. The structure of corynoline. *Chem Pharm Bull.* 1963, 11(10):1306-1312;

Compound 14

Lenfield, J. Karoutil, M. Marsalek, E. Slavik, J. Preininger V. and Simanek. V. *J Med Plant Res.* 1981, 43:161-165.

However, no literature teaches or suggests that these chelidonine-based compounds have K^+ channel blocking effects and anti-arrhythmic effects.

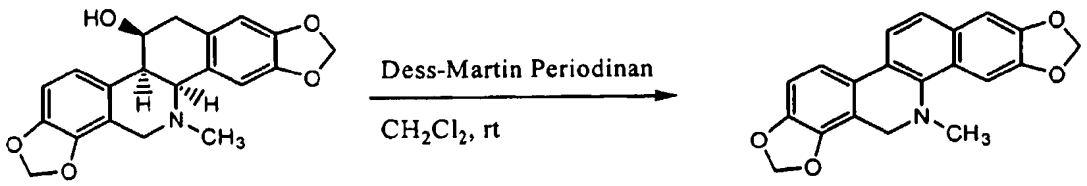
Chelidonine or derivatives thereof used in the present invention can be either purchased commercially, or prepared by the following methods.

In accordance with the method of Bary, D. K., *et al.* (The benzophenanthridine alkaloids. *J Nat Prod* 1984, 47:1-43), *Chelidonium majus* var. *asiaticum* is extracted with methanol. Then, the extract is solvent-fractionated with hexane, dichloromethane, butanol and water. Hexane fraction is selected as an active fraction, and purified by silica gel column chromatography (eluent: dichloromethane/ethylacetate/methanol) to prepare chelidonine or its derivatives.

Further, in accordance with the methods of Takeo, *et al.* (Chiroptische eigenschaften und absolue konfiguration von (+)-14-epicorynolin, (+)-corynolin, (+)-chelidonine und verwandten verbindungen. *Arch Pharm.*(Weinheim) 1984, 317:223-237) and Budabary, *et al.* (The Merck Index, 8th ed. Merck & Co., 1989, 2038-2039), chelidonine is reacted with RCl (wherein R represents alkyl, phenyl, or phenyl substituted with a halogen or lower alkyl), RCOCl(R represents alkyl, phenyl, or phenyl substituted with a halogen or lower alkyl) and chlorosulfonyl isocyanate to prepare chelidonine or its derivatives.

Some compounds used in the present invention can also be prepared in accordance with the following reaction schemes 1 to 5. These schemes are only illustrative of preferred methods for preparing compounds used in the present invention. These schemes, however, are not to be construed as limiting the scope of the present invention.

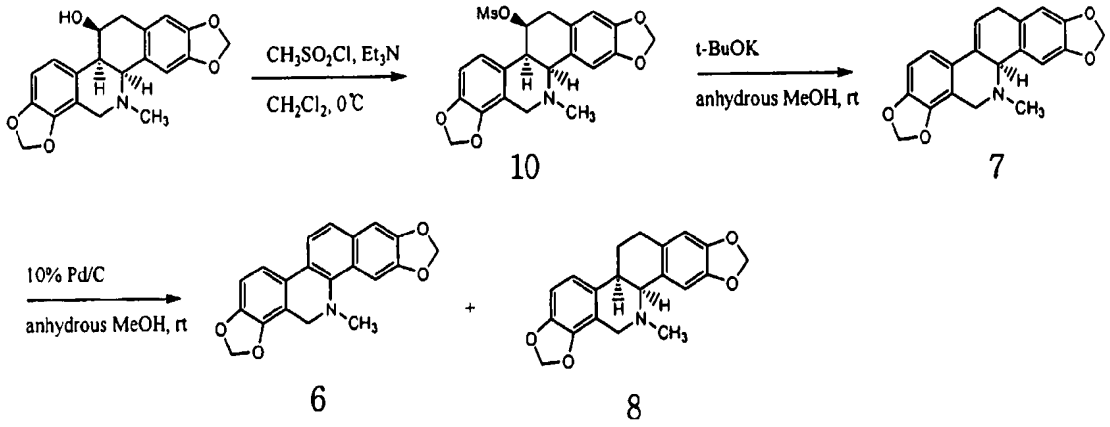
[Scheme 1]



6

By treating chelidonine with Dess-Martin Periodinane oxidizing agent, the secondary alcohol is subjected to dehydration and oxidation to prepare a compound **6**.

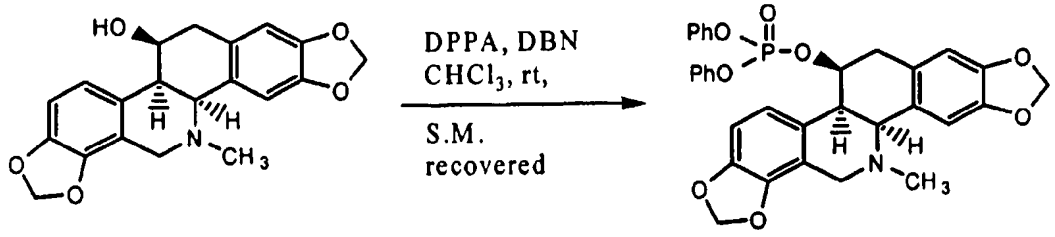
[Scheme 2]



10 7 6 8

Chelidonine is treated with methanesulfonyl chloride in the presence of triethylamine to obtain a mesylate compound **10.** The compound **10** thus obtained is subjected to elimination reaction with potassium t-butoxide to obtain a compound **7.** A double bond present in the compound **7** is reduced with hydrogen in the presence of palladium catalyst to prepare a mixture of compounds **6** and **8**.

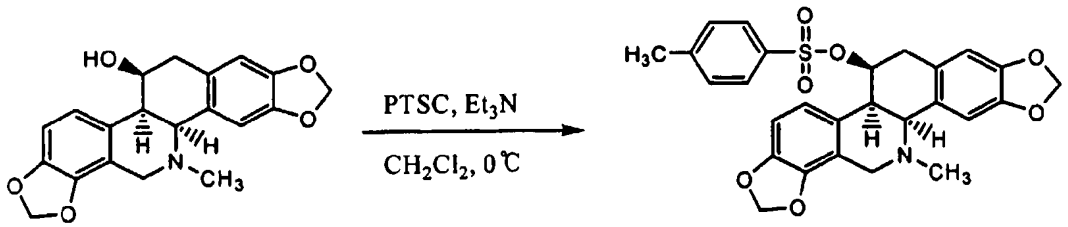
[Scheme 3]



9

Chelidonine is treated with diphenylphosphoryl azide in the presence of 1,5-diazabicyclo[4,3,0]non-5-ene as a base to prepare a phosphoric acid ester compound **9**. At this time, the starting material is recovered from the reaction.

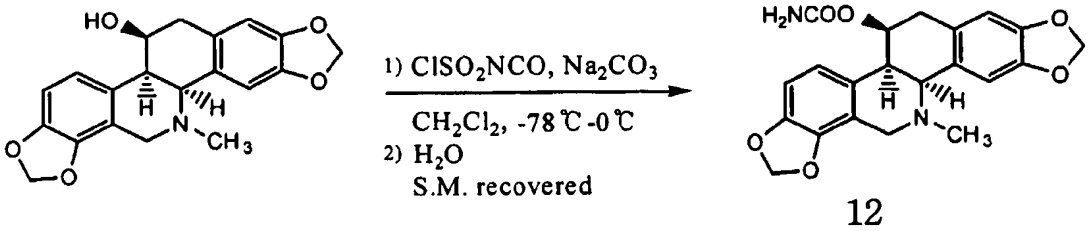
【Scheme 4】



11

Chelidonine is treated with para-toluenesulfonyl chloride in the presence of triethylamine as a base to prepare a sulfone ester compound **11**.

【Scheme 5】



12

Chelidonine is treated with chlorosulfonyl isocyanate in the presence of

sodium carbonate as a base, and then hydrolyzed to prepare a carbamate compound **12**. The starting material is recovered from the reaction.

Examples of pharmaceutically acceptable carriers which may be included in the compositions according to the present invention include excipients, binding agents, lubricants, disintegrating agents, coating agents, emulsifying agents, suspending agents, solvents, stabilizers, absorption agents, water for injection, isotonic agents, etc. The compositions according to the present invention can include at least one selected from these carriers.

The compositions of the present invention can be used as formulations for oral administration or injection, and formulations for oral administration are preferred. Formulations for oral administration can be in the form of granules, tablets, capsules, solutions, etc.

The dosage for the compositions of the present invention can be varied depending upon known factors, such as age, sex, and weight of the patient. The daily dosage is commonly in the range of 10 to 5000mg, and preferably 50 to 1000mg.

The toxicity of chelidonine is 34.6±2.44mg/kg when administered to a mouse intravenously, and that of sanguinarine (compound 14) is 15.9mg/kg when administered to a mouse intravenously and 102.0mg/kg when administered subcutaneously.

The compositions of the present invention can be used as K^+ channel blocking agents and anti-arrhythmic agents, and also used for the treatment of warm-blooded animals such as mouse, dog, rabbit, cat, domestic fowl, etc,.

Brief Description of Drawings

Fig. 1a shows the effect of chelidonine (compound 1) on the hKv1.5 currents expressed in Ltk-cells. The current traces were recorded with a depolarizing pulse of +50 mV from a holding potential of -80 mV;

Fig. 1b shows the effect of sanguinarine (compound 14) on the hKv1.5 currents expressed in Ltk-cells. The current traces recorded with depolarizing step to +50 mV from a holding potential of -80 mV in the absence and the presence of various

concentrations of sanguinarine;

Fig. 1c shows the effect of acetylchelidonine (compound 4) on the hKv1.5 currents expressed in Ltk-cells. The current traces recorded with depolarizing step to +50 mV from a holding potential of -80 mV in the absence and the presence of various concentrations of acetylchelidonine;

Fig. 1d shows the effect of benzoylchelidonine (compound 5) on the hKv1.5 currents expressed in Ltk-cells. The current traces recorded with depolarizing step to +50 mV from a holding potential of -80 mV in the absence and the presence of various concentrations of benzoylchelidonine;

Fig. 1e is concentration-response relationships of hKv1.5 block by chelidonine (compound 1, ○), acetylchelidonine (compound 4, ▲), benzoylchelidonine (compound 5, ■), and sanguinarine (compound 14, ●), respectively. Steady state currents taken at the end of the depolarizing pulse were normalized to control to construct the concentration-response curve;

Fig. 2 shows the voltage-dependent block of hKv1.5 expressed in Ltk- cells by chelidonine (compound 1, 10 μM);

Fig. 3 shows the channel state-dependent block of hKv1.5 expressed in Ltk-cells by chelidonine (compound 1, 10 μM);

Fig. 4a shows representative K^+ current tracings for the effect of chelidonine (compound 1, 10 μM) on the K^+ channel current in human atrial myocytes. K^+ currents were obtained by depolarizing pulse of +50 mV from a holding potential of -80 mV;

Fig. 4b shows the averaged currents obtained in Fig. 4a as determined at the end of the pulse in seven cells;

Fig. 5a shows representative tracings of action potentials in the absence of chelidonine (1 μM) under varied stimulus-frequencies;

Fig. 5b shows representative tracings of action potentials in the presence of chelidonine (compound 1, 1 μM) under varied stimulus-frequencies; and

Fig. 5c shows averaged APD_{90} (action potential duration at 90% repolarization) changes for action potential duration prolongation by chelidonine (compound 1, ○), acetylchelidonine (compound 4, ▲), benzoylchelidonine

(compound 5, ■), and sanguinarine (compound 14, ●), respectively, under varied stimulus-frequencies.

Best Mode for Carrying Out the Invention

The present invention will now be described in more detail with reference to the following examples. However, these examples are given by way of illustration and not of limitation.

Example 1: Preparation of (12bR)-13,14-dihydro-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridine (compound 6)

Dess-Martin Periodinane (25mg, 0.059mmol) was dissolved in anhydrous methylene dichloride (0.2ml), and then stirred at room temperature for 10 minutes. A solution of chelidonine (20mg, 0.054mmol) in anhydrous methylene dichloride (0.1ml) was added dropwise to the reaction solution. After stirring for 30 minutes, diethyl ether (0.9ml) was added to the reaction solution. The reaction solution was added to a mixture of saturated aqueous potassium carbonate solution (0.56ml) and sodium thiosulfate pentahydrate (0.1 g) and then stirred for 15 minutes. The reaction solution was extracted with ethylacetate, washed, dried, and purified by column chromatography (hexane : ethylacetate = 10 : 1) to prepare 6.6mg (37%) of the title compound (compound **6**). At this time, 9mg (45%) of chelidonine was recovered.

R_f= 0.24 (hexane : ethylacetate = 10 : 1)

^{1}H-NMR (500MHz, $CDCl_3$) : δ 7.71 (d, 1H, J=9.0Hz), 7.60 (d, 1H, J=14Hz), 7.50 (d, 1H, J=8.5Hz), 7.32 (d, 1H, J=8.0Hz), 7.27 (s, 1H), 6.87 (d, 1H, J=8.5Hz), 6.07 (s, 2H), 6.06 (s, 2H), 4.21 (s, 2H), 2.64 (s, 3H).

Example 2: Preparation of (12bR)-7, 12b, 13, 14-tetrahydro-13-methyl[1,3]-benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridine (compound 7)

A solution of the compound **10** (20mg, 0.046mmol) in anhydrous methanol (0.3ml) was stirred at room temperature for 10 minutes, and then potassium t-butoxide (15mg, 0.138mmol) was added thereto. The reaction solution was stirred at room temperature for 13 hours. The reaction solution was extracted with ethylacetate,

washed, dried, and purified by column chromatography (hexane : ethylacetate=4 : 1) to prepare 10mg (64%) of the title compound (compound 7).

R_f= 0.22 (hexane : ethylacetate = 4 : 1)

^{1}H-NMR (500MHz, $CDCl_3$) : δ 7.16 (d, 1H, J = 6.0Hz), 7.16 (d, 1H, J = 8.0Hz), 6.74 (d, 1H, J = 8.0Hz), 6.60 (s, 1H), 6.50-6.48 (m, 1H), 5.97 (d, 2H, J = 10Hz), 5.93 (d, 2H, J = 10Hz), 4.56-4.53 (m, 1H), 4.43 (d, 2H, J = 16.5Hz), 3.92 (d, 2H, J = 16.5Hz), 3.59-3.41 (m, 2H), 1.99 (s, 3H).

Example 3: Preparation of [5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridine (compound 8)

10% palladium (1mg) was added to the compound **7** (10mg, 0.030mmol) in anhydrous methanol (0.3ml) and stirred at room temperature for 10 minutes. While feeding with hydrogen, the reaction solution was stirred for 1 hour. The reaction solution was filtered through a pad of celite, concentrated under reduced pressure, and purified by column chromatography (hexane : ethylacetate = 6 : 1) to prepare a mixture of 2mg (20%) of the title compound **8** and 1.8mg (18%) of the compound **6**.

R_f = 0.27 (hexane : ethylacetate = 6 : 1)

^{1}H-NMR (500MHz, $CDCl_3$) : δ 6.93 (s, 1H), 6.75 (d, 1H, J = 8.5Hz), 6.72 (d, 1H, J = 8.5Hz), 6.56 (s, 1H), 5.94 (d, 2H, J = 15Hz), 5.92 (d, 2H, J = 3Hz), 3.94 (d, 1H, J = 16.5Hz), 3.61 (d, 1H, J = 4.0Hz), 3.48 (d, 1H, J = 16Hz), 3.07-3.04 (m, 1H), 2.79-2.67 (m, 2H), 2.40 (s, 3H), 1.91-1.88 (m, 1H).

Example 4: Preparation of {[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridin-6-yl}-diphenylphosphate (compound 9)

1,5-diazabicyclo[4,3,0]non-5-yne (30㎕, 0.243mmol) was added to the solution of chelidonine (30mg, 0.081mmol) in anhydrous chloroform (0.4ml) and stirred at room temperature for 30 minutes. Diphenylphosphoryl azide (27㎕, 0.122mmol) was added to the solution, and stirred for 12 hours. The resulting reaction solution was extracted with ethylacetate, washed, dried, and purified by column chromatography (hexane : ethylacetate = 2 : 1) to prepare 20mg (42%) of the title compound (compound

9). At this time, 11mg (37%) of chelidonine was recovered.

R_f = 0.31 (hexane : ethylacetate = 2 : 1)

^{1}H-NMR (500MHz, $CDCl_3$) : δ 7.41-7.35 (m, 5H), 7.31-7.19 (m, 7H), 6.59 (d, 1H, J = 8.5Hz), 6.37 (s, 1H), 5.90 (s, 2H), 5.88 (d, 2H, J = 23Hz), 5.11-5.05 (m, 1H), 4.10 (d, 2H, J = 5.0Hz), 3.70 (d, 2H, J = 17.5Hz), 3.66 (bs, 1H), 3.44 (d, 2H, J = 17Hz), 2.99 (1H, dd, J = 11, 15Hz), 2.87 (1H, dd, J = 5, 15.5Hz), 2.53 (s, 3H).

Example 5: Preparation of {[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridin-6-yl}-methanesulfonate(compound 10)

Chelidonine (18mg, 0.048mmol) was dissolved in anhydrous methylene dichloride (0.5ml), and then stirred at 0℃ for 10 minutes. Anhydrous triethylamine (8 μℓ, 0.058mmol) and methanesulfonyl chloride (6μℓ, 0.073mmol) were added to the solution, and stirred at room temperature for 6 hours. The resulting reaction solution was extracted with ethylacetate, washed, dried, and purified by column chromatography (hexane : ethylacetate = 2 : 1) to prepare 16mg (77%) of the title compound (compound **10**).

R_f = 0.39 (hexane : ethylacetate = 2 : 1)

^{1}H-NMR (500MHz, $CDCl_3$) : δ 7.21 (d, 1H, J = 8.0Hz), 7.18 (s, 1H), 6.69 (d, 1H, J = 8.5Hz), 6.42 (s, 1H), 5.90 (d, 2H, J = 5.0Hz), 5.88 (d, 2H, J = 15.5Hz), 5.18-5.16 (m, 1H), 4.13-4.11 (m, 1H), 3.75 (s, 1H), 3.73 (d, 2H, J = 12Hz), 3.45 (d, 2H, J = 17Hz), 3.14-3.08 (m, 1H), 3.05 (s, 3H), 2.94 (1H, dd, J = 5.0, 15Hz), 2.54 (s, 3H).

Example 6: Preparation of {[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridin-6-yl}-4-methylbenzenesulfonate (compound 11)

A solution of chelidonine (10mg, 0.027mmol) in anhydrous methylene dichloride (0.3ml) was stirred at 0℃ for 10 minutes. Anhydrous triethylamine (5μℓ, 0.032mmol) and paratoluenesulfonyl chloride (8μℓ, 0.040mmol) were added to the solution, and stirred at room temperature for 12 hours. The resulting reaction solution was extracted with ethylacetate, washed, dried, and purified by column chromatography

(hexane : ethylacetate = 2 : 1) to prepared 8.6mg (63%) of the title compound (compound **11**).

R_f = 0.36 (hexane : ethylacetate = 2 : 1)

^{1}H-NMR (500MHz, $CDCl_3$) : δ 7.86 (d, 2H, J = 8.5Hz), 7.37 (d, 2H, J = 8.5Hz), 7.30-7.26 (m, 1H), 7.20 (s, 1H), 6.66 (d, 2H, J = 8.0Hz), 6.33 (s, 1H), 5.89 (d, 2H, J = 3.5Hz), 5.87 (d, 2H, J = 18Hz), 4.96-4.92 (m, 1H), 4.03 (d, 1H, J = 5.0Hz), 3.67 (d, 2H, J = 17.5Hz), 3.60 (bs, 1H), 3.42 (d, 2H, J = 17.5Hz), 2.98 (1H, dd, J = 11.5, 15Hz), 2.68 (1H, dd, J = 5.0, 15.5Hz), 2.51 (s, 3H), 2.47 (s, 3H).

Example 7: Preparation of {[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridin-6-yl}-carbamate (compound 12)

A solution of sodium carbonate (10mg, 0.097mmol) and chlorosulfonyl isocyanate (6㎕, 0.065mmol) in anhydrous methylene dichloride (0.4ml) was stirred at -78℃ for 10 minutes. A solution of chelidonine (20mg, 0.054mmol) in anhydrous methylene dichloride (0.14ml) was added dropwise to the reaction solution. After the resulting reaction solution was stirred for 1 hour and slowly heated to 0℃, the reaction was quenched with water. The reaction solution was brought to pH > 7 with 3N aqueous sodium hydroxide solution. Subsequently, the reaction solution was extracted with ethylacetate, washed, dried and purified by column chromatography (hexane : ethylacetate = 1 : 1) to prepare 5.4mg (20%) of the title compound (compound **12**). At this time, 4mg (40%) of chelidonine was recovered.

R_f = 0.31 (hexane : ethylacetate = 1 : 1)

^{1}H-NMR (500MHz, $CDCl_3$) : δ 7.26 (d, 1H, J = 8.5Hz), 7.21 (s, 1H), 6.71 (d, 1H, J = 8.5Hz), 6.45 (s, 1H), 5.92 (d, 2H, J = 5.5Hz), 5.90 (d, 2H, J = 14.5Hz), 5.11-5.07 (m, 1H), 4.78 (bs, 2H), 4.15 (bs, 1H), 3.83 (bs, 1H), 3.75-3.80 (m, 1H), 3.50-3.43 (m, 1H), 3.09-3.08 (m, 1H), 3.02 (dd, 1H, J = 5.0, 15Hz), 2.59 (s, 3H).

Experimental Example 1: Effects of chelidonine and derivatives thereof on hKv1.5 channel currents

1) Preparation of cell line expressing a specific ion channel

Cell line selectively expressing a specific cardiac K^+ channel gene was prepared according to the method described in the thesis (Yang, *et al.*, Inhibition of cardiac potassium currents by the vesnarinone analog OPC-18790: comparison with quinidine and dofetilide. *J Pharmacol Exp Ther.* 1997, 280: 1170-1175; Kwak, *et al.*, Phosphorylation is required for alteration of Kv1.5 K^+ channel function by the Kvbeta 1.3 subunit. *J Biol Chem* 1999, 274: 25355-25361).

First, cDNAs for human cardiac K^+ channel genes such as hKv1.5 and HERG were subcloned into pMSVneo which contain dexamethasone-inducible murine mammary tumor virus promoter controlling transcription of the inserted cDNA and a gene conferring neomycin resistance driven by the SV40 early promotor. The cDNA-containing expression vector was transfected into mouse Ltk- cells with lipofectamine. After 24 hours, the cells selection with 0.5μ g/ml of G418 was performed for 2 weeks or until discrete foci formed. Individual foci were isolated, maintained in 0.25μ g/ml of G418, and screened by Norhtern analysis and electrophysiological analysis. Transfected cells were cultured in Dulbeco's modified Eagle medium (DMEM) supplemented with 10% horse serum and 0.25μ g/ml of G418, under 5% CO_2. Transiently transfected cells were cultured in DMEM media supplemented with only 10% horse serum, differently from stable cell lines.

2) Electrical recording

Currents in human atrial myocytes and cell lines were recorded by using the whole cell configuration of the gigaohm-seal patch clamp techniques (Kwak, *et al.*, Phosphorylation is required for alteration of Kv1.5 K^+ channel function by the Kvbeta 1.3 subunit. *J Biol Chem* 1999, 274: 25355-25361). First, electrical signals were amplified with a patch clamp amplifier (Axon Instruments, Axopatch-1D, Foster, USA). Currents were digitized by a signal converter (Digidata 1200, Axon Instruments) and stored on the hard disk of a computer. The micropipette with a resistance of 1~2MΩ (Kimax-51, 1.5-1.8 x 10mm) for current recording, was pulled out by a 2-stage pipette puller (Narishige, PP-83). The intracellular pipette-filling solution for whole cell mode contained 100 mM KCl, 10 mM HEPES, 5 mM K_4BAPTA, 5 mM K_2ATP and 1 mM $MgCl_2$ (pH 7.2). The extracellular solution contained 130 mM NaCl, 4 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES and 10 mM glucose (pH 7.35).

The current traces were recorded with a depolarizing pulse of +50 mV from a holding potential of –80 mV, followed by a repolarizing pulse of –50 mV in the Ltk-cells. To observe the concentration-dependent block of hKv1.5 channel currents by each compound, steady-state currents taken at the end of the depolarizing pulse of +50 mV were normalized to the control obtained in the absence of each compound

Figs. 1a to 1d show the concentration-dependent inhibitory effects of chelidonine (compound 1), sanguinarine (compound 14), acetylchelidonine (compound 4) and benzoylchelidonine (compound 5) on the hKv1.5 channel currents expressed in the Ltk-cells. Fig 1e is concentration-response curves showing the relative steady-state currents obtained at the end of the depolarizing pulse against the concentration of each compound. Data were fitted with a Hill equation.

Figs. 1a to 1e confirmed that these compounds exhibit concentration-dependent inhibitory effects on the hKv1.5 channel currents expressed in Ltk-cells, and that the inhibitory effects varied somewhat depending on the types of derivatives. Especially, chelidonine (compound 1) and acetylchelidonine (compound 4) predominantly blocked the open ion channels. In contrast, sanguinarine (compound 14) slowed the channel activation, and benzoylchelidonine (compound 5) showed the tonic blocking effects.

IC_{50} values (50% inhibitory concentration, μM) of compounds 1~14 on the hKv1.5 channel currents were determined, and Hill values were calculated from the concentration-response curves of the respective compounds using the Hill equation. These results are listed in Table 2.

【Table 2】

IC_{50} and Hill values of chelidonine and derivatives thereof

Compound	IC_{50}(μM)	Hill value
Compound 1	11.5±3.1	1.07
Compound 2	45.2±4.1	1.29
Compound 3	59.3±3.0	1.37
Compound 4	11.3±3.3	1.34
Compound 5	29.3±4.2	1.18
Compound 6	26.4±3.9	1.09
Compound 7	19.9±3.5	0.98
Compound 8	65.6±4.2	1.38
Compound 9	48.6±3.5	1.26
Compound 10	31.3±3.5	1.20
Compound 11	29.6±3.7	1.19
Compound 12	1.9±1.0	1.12
Compound 13	27.5±3.7	1.34
Compound 14	13.0±3.8	1.39

Experimental Example 2: Voltage-dependent block of hKv1.5 channel currents expressed in Ltk- cells by chelidonine

Voltage-dependence of the drugs acting on ion channels is sometimes very useful in evaluating the clinical applications of the drugs.

To quantify the voltage-dependent block of chelidonine (compound 1), the relative steady state current, $I_{chelidonine}/I_{control}$ was plotted as a function of membrane potential (Fig.2). The steady-state currents were obtained at the end stage of 250 ms depolarizing pulses, while increasing the membrane voltage by 10mV from –80 to +60 mV. The dotted line denotes the activation curve of hKv1.5 channel current expressed in Ltk- cells.

As shown in Fig. 2, chelidonine (compound 1) showed a strong voltage-dependence between –30mV and 0mV, but a weak voltage-dependence betweem 0mV and +60mV. δ values between 0mV and +60mV are 0.16±0.01 (n=7) when calculated using the Woodhull equation.

Experimental Example 3: Channel state-dependency of hKv1.5 block by chelidonine.

We tested the channel state-dependency of hKv1.5 block by chelidonine (Fig. 3).

Fig.3 shows the superposition of the tail currents obtained with a depolarizing pulse of +50mV from a holding potential of –80mV, followed by a repolarizing pulse of –50mV under control conditions and in the presence of chelidonine (10 μM).

Under control conditions, the tail currents were rapidly deactivated. In the presence of chelidonine, the initial amplitude of the tail current was reduced, but the subsequent decline of the tail current was slower than that under control conditions, which resulted in a "crossover" phenomenon. This suggests that chelidonine acts as an open channel blocker on the hKv1.5 channel. That is, chelidonine exhibited stronger inhibitory effects on the hKv1.5 channel in an open state than that in a closed state.

Experimental Example 4: Effects of chelidonine on the K^+ current in human atrial myocyte.

Isolation of human atrial myocytes was carried out by the Wang, et al.'s method (Effects of flecainide, quinidine, and 4-aminopyridine on transient outward and

ultrarapid delayed rectifier currents in human atrial myocytes. J Pharmacol Exp Ther 1995, 272: 184-196). Specimens of human right atrial appendage were obtained from the hearts of patients undergoing cardiopulmonary bypass surgery. The procedure for obtaining the tissue was approved by the patient. Samples obtained were quickly immersed in Ca^{2+}-free Krebs Henseleit (KH) solution. The myocardial specimen was chopped and placed in a triangular flask containing 10ml of the Ca^{2+}-free KH solution, followed by agitation for 5 minutes. After removing the supernatant, the tissue was resuspended in 10ml solution containing 200 U/ml collagenase and 4U/ml protease and incubated for 45 min. After removing the supernatant, the tissue was resuspended in a 10ml enzyme-containing solution (in a triangular flask). In order to determine the number and quality of the isolated cells, the medium was examined every 15 min under an inverted microscope. The tissue was incubated until the yield seemed to be maximal. After the incubation, the tissue was transferred to a storing solution. Cells were separated with a pipette, and centrifuged for 5 min under 250g. The sediment was resuspended in a storing solution [composition: 20mM KCl, 10mM KH_2PO_4, 10mM glucose, 70mM glutamic acid, 10mM β-hydroxybutyric acid, 10mM taurine, 10mM EGTA and 0.1% albumin, pH was adjusted to 7.4 with KOH and stored for current recording.

We investigated whether inhibitory effect on the hKv1.5 channel currents by chelidonine is similarly shown for K^+ channel currents of human atrial myocytes, which has been known to express hKv 1.5 channel highly. Human atrial myocytes were isolated from the hearts of patients without any type of arrhythmia undergoing cardiopulmonary bypass surgery. Fig. 4a shows the current traces evoked by depolarizing pulse of +50 mV from a holding potential of –80 mV, then repolarizing to –50 mV. Fig. 4b denotes the averaged steady state currents obtained at the end stage of a depolarizing pulse.

The outward rectifying K^+ currents in human atrial myocytes consist of the rapidly activating and rapidly inactivating current and the delayed rectifying current, as shown in Fig. 4a. Chelidonine (compound 1, 10 μM) decreased the peak amplitude of outward K^+ current in human atrial myocytes and accelerated inactivation process,

resulting in the decrease of the steady state current. Fig. 4b shows that 10μM chelidonine inhibits the steady-state currents at the end of the depolarizing pulse of +50 mV by 41±5% (n=7), compared to the control. Therefore, it was confirmed that inhibitory effect of chelidonine on the hKv1.5 expressed in Ltk- cells is also shown in human atrial myocytes.

Experimental Example 5: Frequency-dependent effects of chelidonine and derivatives thereof on action potentials of rabbit atrial tissue

Action potentials in rabbit atrial tissue were recorded according to the method described in thesis by Kwak, et al. (A newly synthesized benzopyran derivatives, activates the cardiac ATP-sensitive K^+ channel. J Pharmacol Exp Ther 1995, 275: 807-812).

Male New Zealand white rabbits weighing about 2 kg were stunned with a blow on the head and hearts were rapidly excised and transferred to a dissection bath filled with Tyrode's solution saturated with 97% O_2 and 3% CO_2 gas mixture and each dissected tissue was mounted horizontally in a narrow channel of a tissue chamber and continuously superfused with the Tyrode's solution at 37℃. The action potentials were elicited by stimulating the cardiac cells with square pulses (1~5 Hz, 1-ms duration, 20-30% above threshold voltage) by a stimulator. Action potentials were recorded with a 3 M KCl-filled microelectrode (10-20 megaohm) connected to an amplifier (KS-700, WPI, Sarasota, FL, USA), and were displayed on an oscilloscope (Dual beam storage 5113, Tektronix, Beaverton, OR, USA). Tracings on the oscilloscope screen were photographed on a 35-mm film and also recorded on a physiograph (RS 3400, Gould, Cleveland, OH). APD_{90} was used for the comparison of action potential durations. APD_{90} represents the action potential duration at 90% repolarization, relative to the action potential duration at maximum 100% depolarization. Images on the oscilloscope screen were captured on Polaroid camera.

We tested whether the effect of chelidonine on action potentials of rabbit atrial myocytes are frequency-dependent or not.

Figs. 5a (control) and 5b shows the representative tracings of action potentials on varied frequency in the absence (Fig. 5a) or presence (Fig. 5b) of chelidonine

(compound 1), respectively. Fig. 5c shows averaged APD_{90} (action potential duration at 90% repolarization) changes for action potential duration prolongations by chelidonine (compound 1, 0), acetylchelidonine (compound 4, ▲), benzoylchelidonine (compound 5, ■), and sanguinarine (compound 14, ●), respectively, depending on stimulus-frequencies. The relative action potential durations were normalized to the APD_{90} value in the absence of chelidonine at each frequency. At the stimulus frequency of 1, 2, 3, 4 and 5 Hz, 1μM chelidonine increased APD_{90} by 13.0±8.1% (n=5), 14.5±7.9% (n=5), 16.5±2.2% (n=5), 28.2±0.4% (n=5) or 33.0±0.31% (n=5) of the control APD_{90} in the absence of chelidonine at each frequency, respectively. Acetylchelidonine (compound 4), benzoylchelidonine (compound 5) and sanguinarine (compound 14) also increased the action potential durations in the similar manner as chelidonine, but to a lesser extent, compared to that of chelidonine (see, Fig. 5c).

In Figs 5, it was confirmed that chelidonine and derivatives thereof exhibit less antiarrhythmic activity at slow heart rates, but greater antiarrhythmic activity at fast heart rates. Therefore, it is expected that chelidonine and derivatives thereof are useful as ideal antiarrhythmic drugs with fewer side effects.

Preparative Example 1: Tablet

A composition consisting of the following components was formulated into a tablet in accordance with a conventional process.

Tablet composition	
Chelidonine	500.0mg
Lactose	500.0mg
Talc	5.0mg
Magnesium stearate	1.0mg

Preparative Example 2: Capsule

A composition consisting of the following components was formulated into a capsule in accordance with the following process:

First, the sieved chelidonine was mixed with an excipient. A capsule was

prepared by filling gelatin capsule with the mixture.

Capsule composition	
Chelidonine	500.0mg
Starch 1500	10.0mg
Magnesium stearate	100.0mg

Preparative Example 3: Powder

The following components was mixed in accordance with a conventional process. The mixture was filled in an envelope and sealed to prepare a powder.

Powder composition	
Chelidonine	50.0mg
Lactose	100.0mg
Talc	5.0mg

Preparative Example 4: Injection

A composition consisting of the following components was formulated into an injection in accordance with a conventional process. For example, the injection was prepared by filling a 2.0ml ampoule with the composition and by sterilizing it.

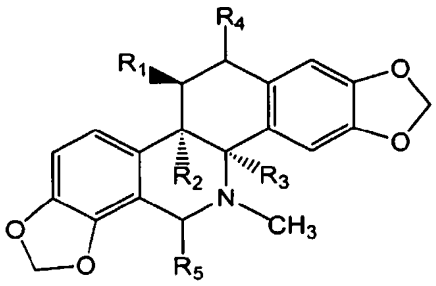
Injection composition	
Chelidonine	50.0mg
Antioxidant	1.0mg
Tween 80	1.0mg
Distilled water for injection	to 2.0ml

Industrial Applicability

The compositions according to the present invention have few problems with regard to safety and toxicity. In addition, the compositions according to the present invention can selectively block K^+ channels expressed in human atrial myocytes, and exhibit less antiarrhythmic activity at slow heart rates but greater activity at fast heart rates. Therefore, the compositions according to the present invention are useful as K^+ channel blockers and antiarrhythmic drugs.

CLAIMS

1. A composition comprising chelidonine or derivatives thereof represented by the following formula **1**, with pharmaceutically acceptable carriers:



(1)

wherein,

R_1 is selected from the group consisting of hydrogen, hydroxy, a lower alkoxy having 1 to 5 carbon atoms, benzyloxy, a lower alkylcarbonyloxy having 1 to 5 carbon atoms, benzoyloxy, a lower alkylsulfonyloxy having 1 to 5 carbon atoms, arylsulfonyloxy, diphenylphosphonyloxy, and -$OCONH_2$;

R_2 is hydrogen or methyl; and

R_3, R_4 and R_5 are each, independently, hydrogen; or

R_1 forms a double bond with R_2 or R_4; or

R_2 forms a double bond with R_3; or

R_5 forms a double bond with the adjacent N atom.

2. The composition of claim 1, wherein R_1 is selected from the group consisting of hydrogen, hydroxy, methoxy, benzyloxy, acetoxy, benzoyloxy, methylsulfonyloxy, 4-methyl-benzenesulfonyloxy, diphenylphosphonyloxy and -$OCONH_2$; and

R_2 is hydrogen or methyl; and

R_3, R_4 and R_5 are each, independently, hydrogen; or

R_1 forms a double bond with R_2 or R_4; or

R_2 forms a double bond with R_3; or

R_5 forms a double bond with the adjacent N atom.

3. The composition of claim 1, wherein the chelidonine or derivative thereof is selected from the group consisting of:

[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridin-6-ol;

[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-6-methoxy-13-methyl[1,3]-benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridine;

[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-6-benzyloxy-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridine;

{[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridin-6-yl}-acetate;

{[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridin-6-yl}-benzoate;

(12bR)-13,14-dihydro-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridine;

(12bR)-7, 12b, 13, 14-tetrahydro-13-methyl[1,3]-benzodioxolo [5,6c]-1,3-dioxolo[4,5-i]phenanthridine;

[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridine;

{[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridin-6-yl}-diphenylphosphate;

{[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridin-6-yl}-methanesulfonate;

{[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridin-6-yl}-4-methylbenzenesulfonate;

{[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridin-6-yl}-carbamate;

[5bR-(5bα, 6β, 12bα)]-5b, 6, 7, 12b, 13, 14-hexahydro-5b, 13-dimethyl[1,3]-benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridin-6-ol; and

13-methyl[1,3]benzodioxolo[5,6c]-1,3-dioxolo[4,5-i]phenanthridinium.

4. The composition of claim 1, wherein the pharmaceutically acceptable carrier includes at least one selected from the group consisting of excipients, binding agents, lubricants, disintegrating agents, coating agents, emulsifying agents, suspending agents, solvents, stabilizers, absorption agents, water for injection and isotonic agents.

5. The composition of claim 1, wherein the composition is formulated into oral administration forms or injection administration forms.

6. The composition of claim 1, wherein the composition is antiarrhythmic drug.

7. The composition of claim 1, wherein the composition is K^+ channel blocking agent.

8. The composition of claim 7, wherein the composition is hKv1.5 channel blocking agent.

9. A method for preventing or treating arrhythmias comprising administering the composition of claim 1.

10. A method for preventing or treating K^+ channel-mediated diseases comprising administering the composition of claim 1.

11. Use of a composition of any one of claims 1 to 3 in the manufacture of a medicament for preventing or treating arrhythmias.

12. Use of a composition of any one of claims 1 to 3 in the manufacture of a medicament for preventing or treating K^+ channel-mediated diseases wherein the medicament has inhibitory effects on K^+ channel.

Fig. 1a

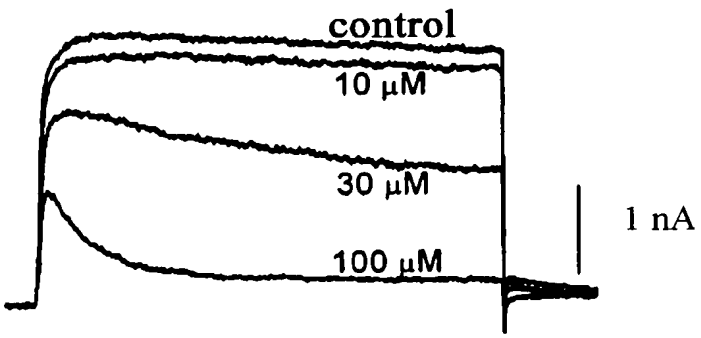


Fig. 1b

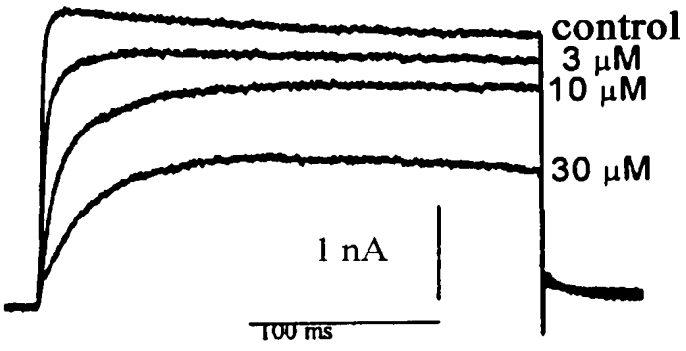


Fig. 1c

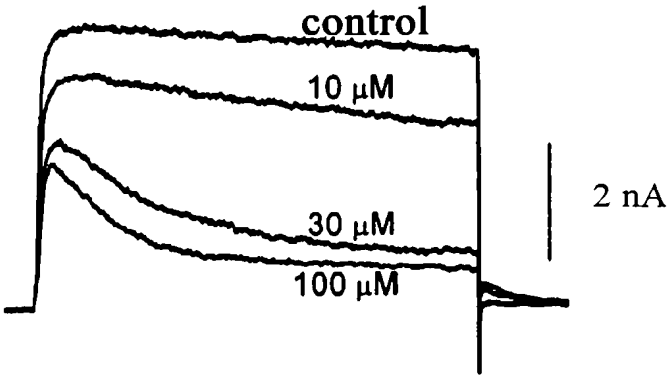


Fig. 1d

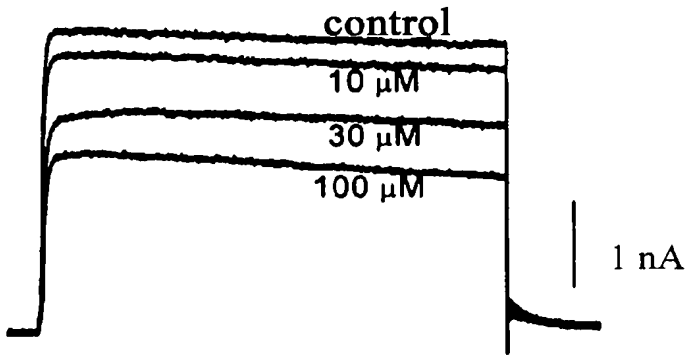


Fig. 1e

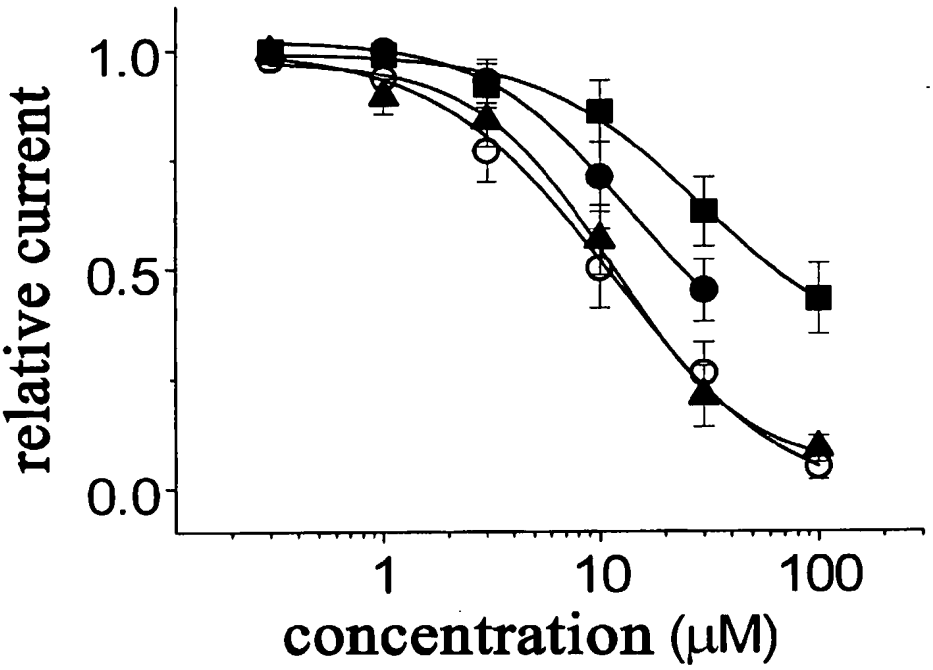


Fig. 2

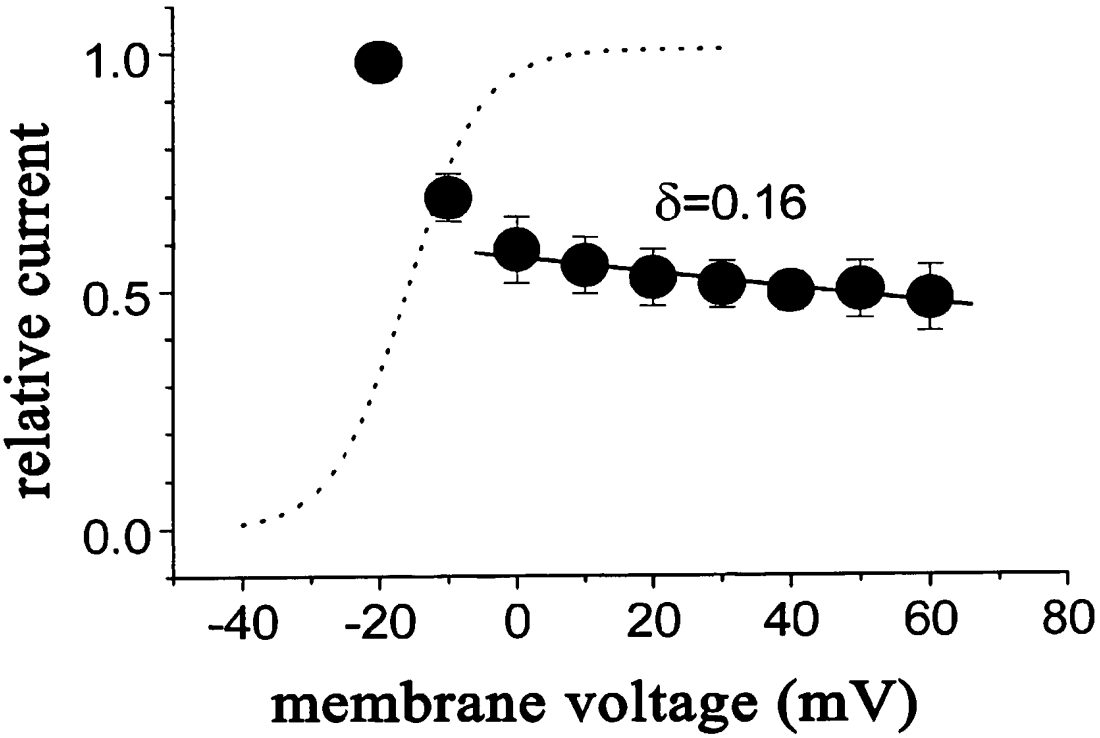

1.0
0.5
0.0
relative current
δ=0.16
-40
-20
0
20
40
60
80
membrane voltage (mV)

Fig. 3

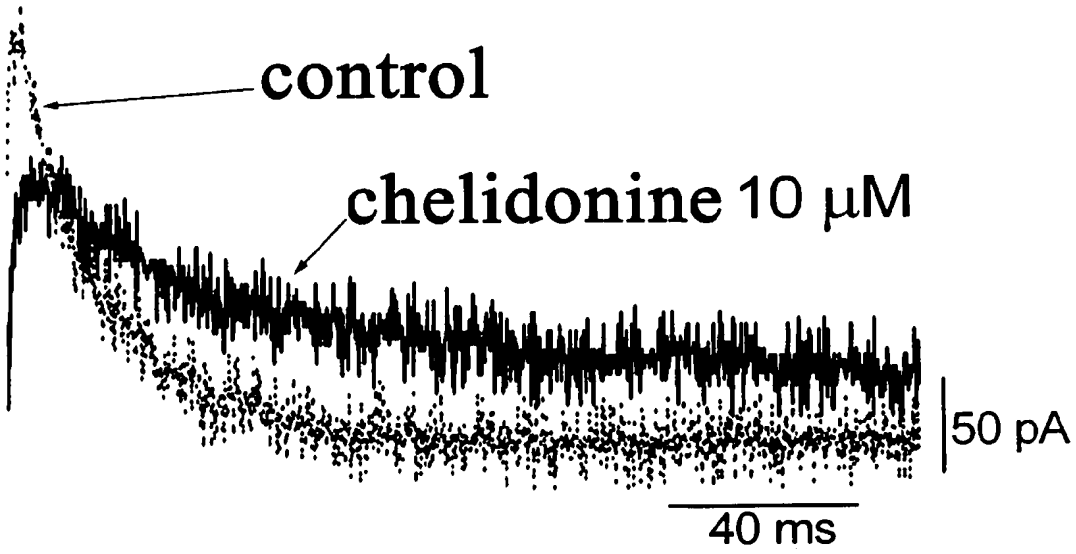


Fig. 4a

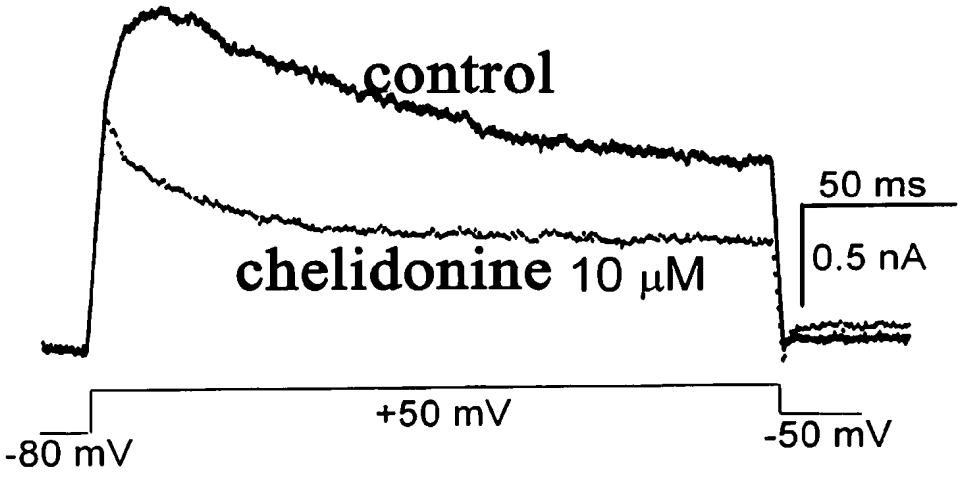


Fig. 4b

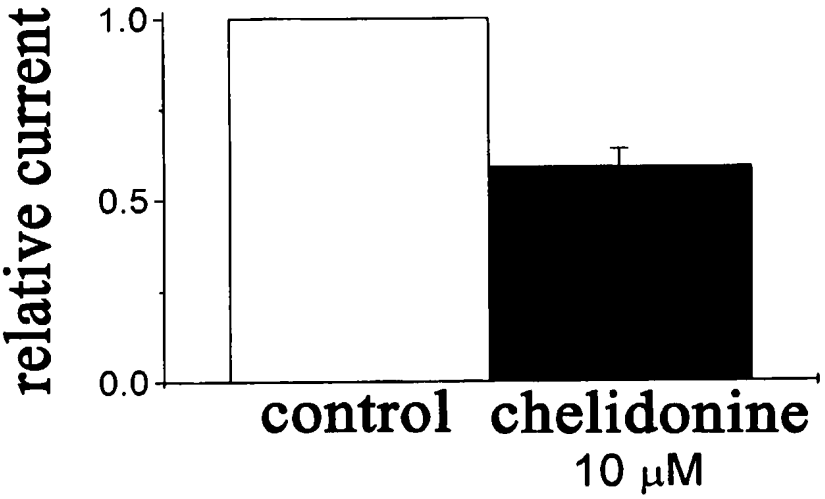


Fig. 5a

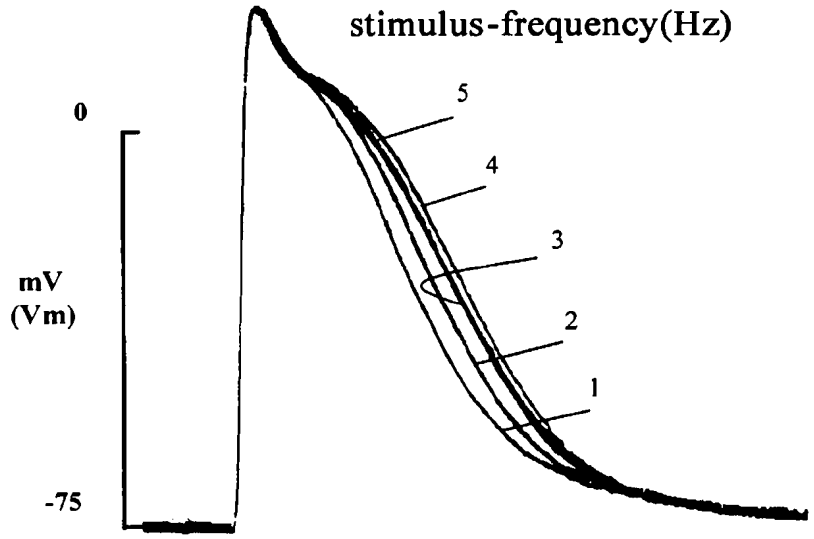


Fig. 5b

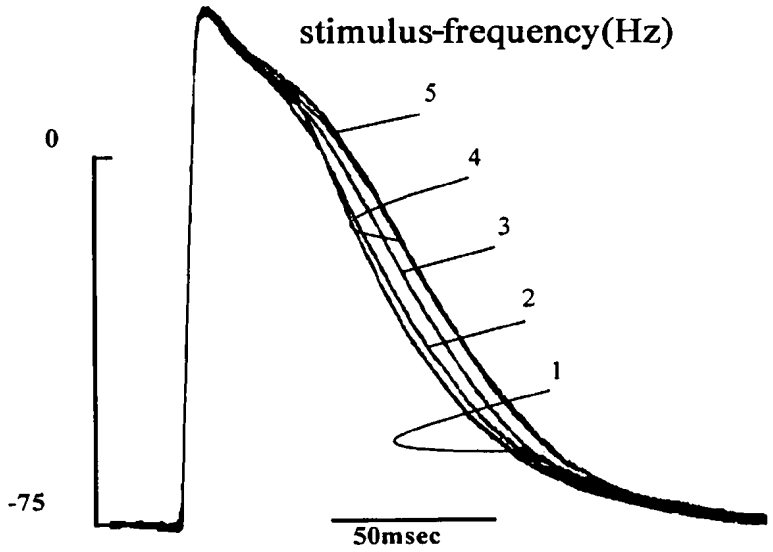
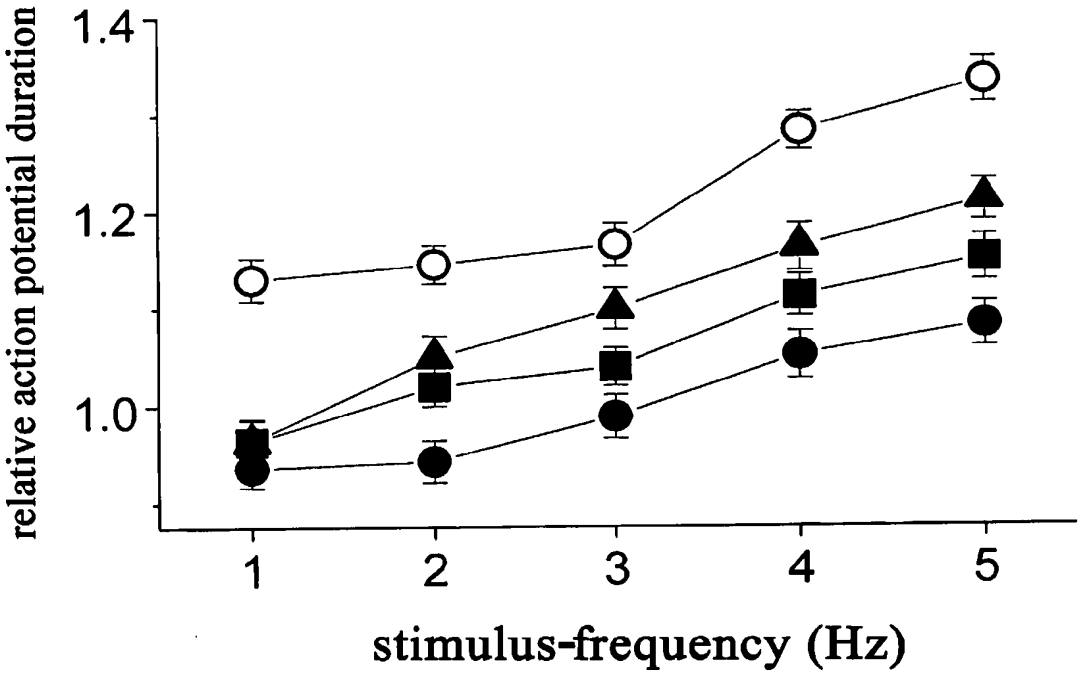


Fig. 5c


relative action potential duration
1.4
1.2
1.0
1
2
3
4
5
stimulus-frequency (Hz)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR02/00865

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 A61K 31/435

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 : A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search terms used)
CA On-Line

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	CN 1237418 A (Liu, Defu, Peop. Rep. China), 08. December 1999, see abstract.	1-5 6-12
X A	WO 0045165 A1 (Cytovia, Inc., U.S.A.), 03. August 2000, see abstract, claims.	1-5 6-12
X A	JP 11302174 A2 (Maruho Co. LTD, Japan), 02. November 1999, see abstract.	1-5 6-12
X A	Hiller K. et al. 'Antispasmodic and relaxant activity of chelidonine, protopine, coptisine, and Chelidonium majus extracts on isolated guinea pig ileum.' In: Planta Medica, 1998, 64(8): pages 758-760, see entire document.	1-5 6-12
X A	Vavreckova C. et al. 'Benzophenanthridine alkaloids of Chelidonium majus, Part 1. Inhibition of 5- and 12-lipoxygenase by a non-redox mechanism.' In: Planta Medica, 1996, 62(5): pages 397-401, see entire document.	1-5 6-12
X A	Vavreckova C. et al. 'Benzophenanthridine alkaloids of Chelidonium majus, Part 2. Potent inhibitory action against the growth of human keratinocytes.' In: Planta Medica, 1996, 62(6): pages 491-494, see entire document.	1-5 6-12

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevence
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevence; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 SEPTEMBER 2002 (11.09.2002)	12 SEPTEMBER 2002 (12.09.2002)

Name and mailing address of the ISA/KR
Korean Intellectual Property Office
920 Dunsan-dong, Seo-gu, Daejeon 302-701,
Republic of Korea
Facsimile No. 82-42-472-7140

Authorized officer
HAN, Hyung Mee
Telephone No. 82-42-481-5601

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR02/00865

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 9, 10
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 9 to 10 is directed to a method of treatment of the human/animal body, the search has been carried out on the basis of the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to part of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Search Authority found multiple inventions in this international application, as follows:

1. [ ] As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be established without effort justifying an additional fee, this Authority did not invite payment of any addition fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR02/00865

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
CN 1237418 A	08. 12. 1999	None	
WO 0045165 A1	03. 08. 2000	None	
JP 11302174 A2	02. 11. 1999	None	